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Environmental conditions at the Last Interglacial (Eemian) site Neumark-Nord 2, Germany inferred from stable isotope analysis of freshwater mollusc opercula

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Mollusc biogenic carbonates are valuable records of past environmental conditions. In particular, carbonate oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) stable isotopes can be used to reconstruct different physical and chemical parameters, according to the different genera used (marine, freshwater or terrestrial). The Last Interglacial (early Eemian) palaeolake of Neumark-Nord 2 (NN2), Germany provides an excellent example of a Neanderthal archaeological site with abundant freshwater carbonate remains. As in other European contexts, one of the most abundant species is *Bithynia tentaculata.* In order to provide a robust regional baseline for the interpretation of the archaeological data, this study includes a calibration phase on modern *B. tentaculata* opercula. The results indicate that these calcitic structures are likely to be subjected to a growth slowdown/cessation during summer, which influences their geochemistry, reflecting mainly the water properties of the rest of the year. This modern calibration, together with the existing palaeoenvironmental reconstructions developed for NN2 (e.g. pollen data), represents a valuable opportunity to establish *B. tentaculata* opercula as reliable environmental proxies applicable to several other freshwater contexts. The isotope data of the NN2 opercula, in agreement with the pollen record, indicate that the major archaeological horizon was formed during a rather wet period

24 and potentially in a semi-forested environment. However, human occupation occurred also during

25 drier phases at the site and within a wide temperature range, indicating the absence of restricted

26 environmental preferences by the local Neanderthal groups.

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50 Remains of freshwater molluscs, and in particular *B. tentaculata*, are abundant and well preserved

51 throughout the Quaternary sedimentary record across Europe (Preece 2001). Recent advances in

52 biomolecular techniques and biomineral understanding have resulted in the application of amino

53 acid racemization analyses of these biominerals for dating (Penkman *et al*. 2008, 2011). However,

54 only few studies have previously used these materials as palaeoenvironmental records (Filippi *et*

55 *al.* 1997; Hammarlund *et al.* 1999; Anadón *et al.* 2006).

56   Given that the *B. tentaculata* habitat range includes closed and slow-running water bodies,

57 shells and opercula are often found in interglacial palaeolake infills (Anadón *et al.* 2006; Waghorne

58 *et al.* 2012; Milano & Szymanek 2019). Previous studies suggested that *B. tentaculata* opercula

59 constitute suitable environmental records and that their oxygen and carbon stable isotope

60 compositions are useful proxies for water temperatures and moisture regimes, respectively (Milano

61 & Szymanek 2019). For instance, oxygen incorporation by freshwater molluscs is influenced

62 mainly by the water oxygen $\delta^{18}O$ and temperature (Anadón *et al.* 2006; Waghorne *et al.* 2012).

63 Furthermore, the carbonate $\delta^{13}C$ is influenced by the water DIC (dissolved inorganic carbon),

64 which, in turn, is controlled by water inflow and evaporation related to the moisture extent of the

65 environment (Leng & Marshall 2004 and references therein).

66   High-resolution climatic reconstructions of interglacial periods, and especially of the Last

67 Interglacial, are valuable tools for the palaeoecological interpretation of Pleistocene human-

68 environment interactions. Evidence of human activity during the Last Interglacial in central Europe

69 has been attested to by the discovery of several sites in northern and central Germany such as

70 Gröbern, Grabschütz, Weimar, Neumark-Nord, Rabutz, Lehringen and Stuttgart-Untertürkheim

71   (Roebroeks *et al.* 1992; Gaudzinki-Windheuser & Roebroeks 2011). Among them, Neumark-Nord

72   2 (NN2) stands out: the record includes not only abundant evidence for hominin activity

73   (Gaudzinski *et al.* 2014; Pop 2014), but it also contains several organic and inorganic materials

74   useful for palaeoenvironmental reconstructions, including pollen (Sier *et al.* 2011; Bakels 2014;

75   Wansa *et al.* 2014), sediment (Mücher 2014) and mammalian biominerals (Britton *et al.* 2019).

76   The aim of this study is to produce additional evidence for palaeoenvironmental conditions at NN2.

77   The abundance of *B. tentaculata* opercula throughout the *Hauptprofil 7* (HP7) sequence, together

78   with the existence of numerous other environmental proxies, represents a unique opportunity to

79   achieve such objective. In addition, the present study includes a modern calibration of *B.*

80   *tentaculata* $\delta^{18}O$ and $\delta^{13}C$ based on a year-long collection and environmental monitoring at

81   Hundewasser (near Leipzig, Germany). This part of the study was designed to further refine our

82   understanding of the freshwater biogenic carbonates as environmental records.

83

84

85   Study site: Neumark-Nord 2 (NN2)

86   Neumark-Nord is located ~ 35 km west of Leipzig in Saxony-Anhalt, Germany (51°19′28″ N,

87   11°53′56″ E). The first excavations uncovered the Neumark-Nord 1 basin and subsequently, less

88   than 200 meters northeast, a second basin, Neumark-Nord 2, was discovered in 1996 (Fig. 1;

89   Gaudzinski-Windheuser *et al.* 2014). The NN2 basin infill revealed archaeological deposits related

90   to the Last Interglacial period, with the lake estimated to have started forming in the last part of

91   Saalian and to have lasted throughout the Eemian (Sier *et al.* 2011). In July 2007, the NN2 profile

92   HP7, located in the deep part of the infill, was sampled at 5-cm intervals with the deepest sample

93   at 595 cm from the top of the section. The lithostratigraphic succession was recorded by Mücher

94 (2014). The pollen record was documented along the entire HP7 profile and allowed a

95 reconstruction of the (local) vegetational change of the Eemian Interglacial (Sier *et al.* 2011;

96 Bakels 2014; Wansa *et al.* 2014). Numerous archaeological finds, including faunal and lithic

97 assemblages, were excavated from the margin of the basin in different units that are well correlated

98 to the HP7 profile. Based on multidisciplinary analyses of the finds, previous studies suggested

99 that the area around NN2 was used primarily for food processing and flint tool production (Kindler

100 *et al.* 2014; Pop 2014). Ongoing studies are focused on correlating the fine-grained archaeological

101 data from NN2 to the record obtained during rescue excavations of the neighbouring, much larger,

102 lake of Neumark-Nord 1 (e.g. Gaudzinski-Windheuser *et al.* 2018).

103   Along the NN2 HP7 stratigraphic succession freshwater molluscs dominated the mollusc fauna

104 (Strahl *et al.* 2010; Kuijper 2014). Especially abundant were the species of *B. tentaculata* (used in

105 this study), *Valvata piscinalis, Radix ovata, Anisus spirorbis, Gyraulus acronicus* and *Gyraulus*

106 *crista*. The mollusc fauna indicated calm, shallow freshwater conditions (Kuijper 2014).

107

108
109 Material and methods

110 *Bithynia tentaculata and modern sampling*

111 *B. tentaculata* (Linnaeus, 1758) is a prosobranch gastropod living in European slow-running rivers

112 and lakes (Schäfer 1953; Frömming 1956). The snail has a short lifespan of about 1-3 years, with

113 sexual maturity reached within the first 12 months of growth (Tashiro & Colman 1982; Richter

114 2001). It is characterized by a small shell (up to 13 mm) with five whorls and an upside-pointing

115 operculum (Clarke 1981; Jokinen 1992; Welter-Schultes 2012). As for all molluscs, the

116 biomineralized structures are deposited throughout the animal's life. The shell is composed of

117 aragonite while the operculum is made of calcite (Filippi *et al.* 1997; Anadón *et al.* 2006).

118   For the modern component of this study, collection of live specimens was performed on a

119 monthly basis for a period of one year from November 2017 to November 2018. Molluscs were

120 collected from the small river Hundewasser in Lützschena near Leipzig, Germany (51°22′37.3″N,

121 12°16′50.7″E) ca. 30 km east of NN2 (Fig. 1). The molluscs (N = 39) were collected using small

122 fishing nets at a depth of 50-100 cm. Juveniles and small specimens were avoided. On average,

123 the opercula collected were 4.6 ± 0.2 mm long and 3.2 ± 0.1 mm wide.

124   The river is about 8 m wide and 1 m deep and it is characterized by extremely low current

125 levels. Its banks are vegetated with abundant aquatic macrophytes. Water samples were collected

126 and stored in 40-mL glass bottles with no headspace at ~ 4 °C until measurement. Air and water

127 temperatures were measured during collection using a Voltcraft DT-300 thermometer.

128

129 *Sample preparation and analysis*

130 The soft tissues of the modern molluscs were discarded immediately after collection. In order to

131 remove foreign material, the carbonates were immersed in 3 vol % NaOCl for 2 hours and

132 ultrasonically washed using deionized water for circa 3-5 minutes. After being air-dried for 24

133 hours, the opercula (N = 39) were individually crushed using an agate mortar and pestle.

134   The opercula from NN2 were bleached, washed and crushed following the same protocol as the

135 modern specimens. A total of 107 opercula were analysed from the NN2 collection. On average,

136 three opercula were selected from 41 5-cm mollusc-rich intervals in the HP7 stratigraphic

137 succession. The analysed opercula came from units (and archaeological levels): 5, 6 (NN2/3), 7, 8

138 (NN2/2b), 9 (NN2/2a), 10, 11 (NN2/1c), 17 (NN2/1a) and 18.

139   The stable isotope analyses of the carbonates were performed at two laboratories, the Iso-

140   Analytical laboratory in Crewe, United Kingdom and the Department of Human Evolution, Max

141   Planck Institute for Evolutionary Anthropology in Leipzig (henceforth MPI-EVA), Germany. At

142   the Iso-Analytical laboratory, carbonate powder samples were digested in He-flushed borosilicate

143   exetainers using a water-free phosphoric acid. The released CO_2 gas was then measured in

144   continuous flow mode with a Europa Scientific 20-20 gas source isotope ratio mass spectrometer

145   (CF-IRMS). At MPI-EVA, samples were digested at 70 °C and the CO_2 gas was then measured

146   with a ThermoFisher MAT 253 Plus IRMS linked to a Kiel IV automated carbonate preparation

147   device. At Iso-Analytical, stable isotope ratios were calibrated against an IA-R022 calibrated NBS-

148   19 ($\delta^{13}C = +1.95‰$; $\delta^{18}O = -2.20‰$), whereas at MPI-EVA, data were calibrated against an IAEA-

149   603 calibrated Carrara marble ($\delta^{18}O = -1.64‰$; $\delta^{13}C = +1.87‰$). Results are reported in per mil

150   (‰) relative to the Vienna Pee-Dee Belemnite (VPDB) standard. At Iso-Analytical, the average

151   precision error (1σ; computed from eight injections per sample) was better than 0.05‰ for both

152    $\delta^{18}O$ and $\delta^{13}C$. At MPI-EVA, the average precision error (1σ; computed from eight measurements

153   per sample) was better than 0.05‰ for $\delta^{18}O$ and 0.03‰ for $\delta^{13}C$, and the long-term accuracy based

154   on IAEA-603 samples measured blind (n = 268) was better than 0.05‰ for $\delta^{18}O$ and 0.03‰ for

155    $\delta^{13}C$.

156   The oxygen stable isotope analyses of the water collected on monthly basis from Hundewasser

157   were performed at the Iso-Analytical laboratory in Crewe, United Kingdom. The samples were

158   measured by continuous flow isotope ratio mass spectrometry using a Europa Scientific ANCA-

159   GSL and 20-20 IRMS. The analyses were performed in duplicate by the equilibration technique.

160   The samples were measured against three reference standards. The first standard being IA-R063

161   with $\delta^{18}O$ V-SMOW $= -0.41‰$, the second IA-R065 with $\delta^{18}O$ V-SMOW $= -33.57‰$ and the third IA-

162　R064 with $\delta^{18}O$ $_{V\text{-SMOW}}$ = −12.34‰. All three standards are traceable to the primary reference

163　standards V-SMOW2 (Vienna-Standard Mean Ocean Water) and V-SLAP2 (Vienna-Standard

164　Light Antarctic Precipitation) distributed by the IAEA. All the modern isotope and environmental

165　data can be found in the Supplementary Information (Table S1).

166　In order to test the water isotopic equilibrium deposition of *B. tentaculata* opercula, the

167　measured $\delta^{18}O$ values were compared to the expected $\delta^{18}O$ calculated using the following

168　fractionation equation:

169　$T = 15.70 - 4.36\ (\delta^{18}O_c - \delta^{18}O_w) + 0.12\ (\delta^{18}O_c - \delta^{18}O_w)^2$　　　　　　　　(1)

170　(Hays & Grossman 1991) where T = water temperature; $\delta^{18}O_c$ = calcite $\delta^{18}O$; $\delta^{18}O_w$ = water $\delta^{18}O$.

171　For the fossil material from NN2, to standardize the dataset and to enhance long-term isotope

172　trends, departures ("deviations" or "anomalies") in the values of the single specimens from the

173　$\delta^{18}O$ and $\delta^{13}C$ overall averages of the sequence were calculated. The $\delta^{18}O$ and $\delta^{13}C$ 2-sigma

174　standard deviations within the monthly modern datasets (0.7‰ for both isotope ratios) were used

175　as significance thresholds in the interpretation of the archaeological results. The isotope data from

176　NN2 can be found in the Supplementary Information (Table S2).

177

178

179　# Results

180　## *Modern water and carbonate data*

181　At Hundewasser, the seasonal water temperatures fluctuated between 0.6 and 21.7 °C, with a

182　similar variation for the recorded air temperatures (from −4.2 to 21.2 °C; Fig. 2A,B). In association

183　with these variations, the water $\delta^{18}O$ ($\delta^{18}O_w$) showed a variance range of 1.6‰, with more negative

184　values in winter and more positive ones during summer (Fig. 2B). The opercula showed relatively

185 narrow seasonal variances in their isotope values of 0.7‰ for δ^{18}O and 0.9‰ for δ^{13}C (Fig. 2C).

186 Significant offsets occur between measured and expected δ^{18}O monthly values (calculated using

187 Eq. (1)), with rather flat data distributions compared to the expected value distributions (Fig. 3A).

188 The largest offsets occurred during the summer months of June, July and August, suggesting a

189 growth slowdown/cessation during this time of the year (Fig. 3A). The expected average δ^{18}O

190 value (−5.9±1.4‰) using the annual dataset resulted lower than the measured value (−5.4±0.2‰;

191 Fig. 3B). This offset decreased (from 0.5‰ to 0.3‰) when the period of slow growth was excluded

192 from the average calculations, with an expected δ^{18}O value of −5.2±1.0‰ and a measured δ^{18}O

193 value of −5.5±0.3‰ (Fig. 3B). Likewise, the average reconstructed water temperature using the

194 whole annual dataset was calculated to be 7.7±1.8 °C, about 2 °C colder than the measured one

195 (9.8±7.4 °C; Fig. 3C). By excluding the period of slow growth, the reconstructed water temperature

196 (7.4±1.9 °C) and the measured one (6.7±5.1 °C) showed an offset of 0.7 °C.

197

198 *Oxygen isotopes of B. tentaculata opercula from NN2*

199 Compared to the average opercula δ^{18}O values of modern specimens, the archaeological δ^{18}O

200 values were from 2.5‰ to 6.5‰ higher. Along the HP7 profile, the geochemical composition of

201 *B. tentaculata* opercula showed a certain degree of variation. The average δ^{18}O value varied among

202 the different stratigraphic units (Fig. 4A), with the most negative values recorded in units 6, 10

203 (respectively −1.7±1.1‰ and −2.3±1.1‰) and 18 (with only two specimens; −2.9±2.0‰) and the

204 most positive values recorded in units 5 and 17 (resp. 0.8±0.6‰ and 0.9±1.9‰). Data in units 6,

205 7, 8 and 17 recorded the largest δ^{18}O variances due to the large spread among the opercula (Fig.

206 4A). In unit 6, the high variance was due to the particularly low δ^{18}O values of the opercula at 545-

207 550 cm and to the particularly high values of the specimens at 550-555 cm (Fig. 5A). In unit 7, the

208   spread of the data was likely affected by the discontinuous sampling of opercula for the unit.

209   Within unit 8, high $\delta^{18}O$ values occurred in the lower part but they decreased toward lower values

210   in the central part of the unit and then they showed a tendency towards high values in the final

211   parts of the unit (Fig. 5A). Furthermore, unit 17 showed a clear tendency toward lower values in

212   the upper part (Fig. 5A).

213   When considering the deviations from the profile $\delta^{18}O$ average, some data resulted to be smaller

214   than the threshold of 0.7‰ (based on the 2σ standard deviations within the monthly modern

215   dataset). Only deviations above this threshold were considered significant for the

216   palaeoenvironmental reconstructions and therefore further discussed (Fig. 6A). Warm (positive)

217   anomalies were observed at the end of unit 5, at the beginning of unit 7 and 8 (NN2/2b) and, to a

218   lesser degree within unit 11 (NN2/1c). Exceptionally positive anomalies were observed at the

219   beginning of unit 17 (NN2/1a). Cold (negative) anomalies were observed at the end of unit 6

220   (NN2/3), 7, 8 (NN2/2b) and, to a lesser degree in unit 9, at the end of unit 10 and in the central

221   part of unit 11 (NN2/1c). A rather large negative anomaly was also observed at the beginning of

222   unit 18 (Fig. 6A).

223

224   *Carbon isotopes of B. tentaculata opercula from NN2*

225   As with oxygen isotope composition, the archaeological $\delta^{13}C$ values were from 5.6‰ to 6.8‰

226   higher than the values of modern specimens. The average $\delta^{13}C$ values varied among the different

227   units (Fig. 5B), with the most negative values recorded in units 8 and 10 (resp. −6.1±0.9‰ and

228   −5.4±1.4‰) and the most positive values recorded in units 7, 11 and 17 (resp. −4.5±1.0‰, −4.1±

229   1.2‰ and −4.5±1.94‰). Likewise for $\delta^{18}O$ data, large $\delta^{13}C$ variances were recorded in units 8 and

230   17 (Fig. 5B). As for unit 8, the variance was primarily associated with exceptionally high $\delta^{13}C$

231   values at depth 430-435 cm, contrasting with the rest of the unit characterized by lower values. In

232   unit 17, the $\delta^{13}C$ showed a tendency toward lower values in the uppermost parts. The same trend

233   was visible within unit 9 (Fig. 5B).

234   When considering the significant deviations (>0.7‰) from the profile $\delta^{13}C$ average (Fig. 6B),

235   dry (positive) anomalies were observed at the end of unit 5, at the beginning of units 6 (NN2/3), 7

236   and 17 (NN2/1a), in unit 11 (NN2/1c). Wet (negative) anomalies were observed at the beginning

237   of units 5, 6 (NN2/3) and 7, through most of unit 8 (NN2/2b) and at the end of unit 17 (NN2/1a).

238

239

240   Discussion

241   *Modern calibration of B. tentaculata*

242   The results of modern *B. tentaculata* specimens demonstrated the potential of the opercula $\delta^{18}O$

243   values to be proxies for water temperature, with an error of less than 1 ˚C. Due to the small size of

244   the opercula, the sampling technique used did not ensure the right spatial resolution to resolve

245   seasonal isotopic fluctuations and therefore sub-annual temperature reconstructions. Other

246   analytical techniques, such as SIMS (Secondary Ion Mass Spectrometry) and SHRIMP (Sensitive

247   High Resolution Ion Microprobe), would be needed to achieve a higher temporal resolution of the

248   data. Although the $\delta^{18}O$ seasonal variability in the opercula was limited, it was possible to identify

249   a period of potential growth slowdown/cessation during the warmest months of summer (Fig. 3A).

250   Generally, in bivalves, the main shell growth coincides with the warm season (Richardson 2001).

251   However, several studies previously reported cases of summer growth cessation (Ramón 2003;

252   Milano *et al.* 2017). Waghorne *et al.* (2012) investigated the growth of *B. tentaculata* opercula

253   from southern Great Britain and they estimated the growing season to be between May and

254   October. The different growing season recorded here is likely related to the animal's exposure to

255   different environmental factors such as food availability, temperature and water conditions

256   (Witbaard *et al.* 1997). Furthermore, *B. tentaculata* is known to spawn between May and July

257   (Richter 2001). The observed slow-down in opercula formation can be a result of energy

258   reallocation from growth to reproduction, which was previously observed in several other mollusc

259   species (Sato 1995; Purroy *et al.* 2018). Our results indicated that *B. tentaculata* $\delta^{18}O$ values

260   reflected well the water temperature throughout the year with the exception of about three months

261   during summer. When applied to palaeoenvironmental studies, this temporal window has to be

262   taken into account.

263   Although the modern data are rather promising, the application of the opercula $\delta^{18}O$ as

264   quantitative palaeothermometer for fossil remains has to be considered with caution. The

265   superficial $\delta^{18}O$ value of freshwater bodies is known to reflect the rainfall $\delta^{18}O$ value, which varies

266   primarily with latitude and altitude (Craig 1961; Bowen & Wilkinson, 2002). At a specific

267   location, the rainfall $\delta^{18}O$ is mainly controlled by air temperature and, in the tropical regions, by

268   the amount of precipitation (Dansgaard 1964). However, the size and type of water body

269   significantly affects the relationship between the water $\delta^{18}O$ and rainfall $\delta^{18}O$ (Darling *et al.* 2003).

270   In fossil contexts, in which the water $\delta^{18}O$ value is not known, a quantitative temperature

271   reconstruction is not advisable, especially when applied to lacustrine environments. Open and

272   large lakes have different hydrological properties compared to small and closed ones, whose $\delta^{18}O$

273   water can be significantly affected by evaporative processes (Darling *et al.* 2003). For this reason,

274   only qualitative temperature reconstructions are recommended (Waghorne *et al.* 2012). Our results

275   from NN2 confirmed that hydrological characteristics significantly influence water and carbonate

276   stable isotope signature. The $\delta^{18}O$ difference observed between modern and archaeological

277 specimens was too large to be simply explained by changes in water temperature, suggesting that

278 the stable isotope composition of the NN2 lake was rather different than the modern Hundewasser.

279 A previous rainfall $\delta^{18}O$ value estimation based on the oxygen isotope analysis of *Equus* tooth

280 enamel from NN2 showed similar values to today's average, with $\delta^{18}O$ precipitation values

281 calculated from main find level NN2/2b being -9.1±1.1 ‰, for example (Britton et al. 2019). This

282 indicates that the differences observed in the opercula was likely related to fractionation processes

283 in the lake water itself. Considering the small size of the lake, NN2 might have been affected by

284 evaporative processes inducing the lake water and therefore the carbonates to have higher $\delta^{18}O$

285 values. The discrepancy between the two types of environments (NN2 and Hundewasser) could

286 have been reduced by collecting modern specimens from a water body similar to NN2. However,

287 several field inspections and personal communications with local malacologists, revealed that the

288 modern distribution of *B. tentaculata* in Saxony is largely confined in small rivers and channels.

289 Therefore, a modern collection from lakes proved unfeasible.

290 As for the $\delta^{18}O$ values, the $\delta^{13}C$ modern variability was low (Fig. 2). Biogenic carbonate $\delta^{13}C$

291 values in lacustrine environments are known to be mainly related to the $\delta^{13}C$ values of the water

292 DIC and therefore water inflow and evaporation processes (Milano & Szymanek 2019 and

293 references therein). In particular, when evaporation exceeds water inflow, the water DIC $\delta^{13}C$ is

294 more enriched, with consequential higher values in the biogenic carbonate $\delta^{13}C$ (Leng & Marshall

295 2004 and references therein). Previous works on *B. tentaculata* suggest that the $\delta^{13}C$ of this species

296 can be used as an evaporation/inflow proxy (Hammarlund *et al.* 2003; Anadón *et al.* 2006; Milano

297 & Szymanek 2019). Hundewasser is a flowing water body with percolation of groundwater

298 recharge. This, together with the small opercula size and consequent isotope signal averaging,

299 could explain the limited variability of the opercula $\delta^{13}C$ values throughout the year. On the other

300   hand, closed hydrological systems, especially when small, are significantly more sensitive to

301   evaporative processes. As for oxygen, $\delta^{13}C$ values of specimens from NN2 were significantly

302   higher than the Hundewasser opercula, supporting the hypothesis that the local water isotope

303   composition would have been significantly different than modern running systems. However,

304   records from evaporation-sensitive settings such as NN2 might offer important insights into the

305   humidity variations at a local scale. For the $\delta^{18}O$ values, the larger sensitivity to evaporation may

306   bias the interpretation of the isotopic signal purely based on water temperature. Therefore, the

307   temperature cannot be reconstructed in a quantitative way and the results have to be considered

308   with caution.

309

310   *Palaeoenvironmental reconstruction of NN2*

311   *B. tentaculata* opercula excavated from NN2 (HP7) indicated that, within the Eemian Interglacial,

312   variations in the local environmental conditions occurred. Two important issues have to be

313   considered when interpreting the opercula NN2 isotopic record: (i) the opercula, as all biogenic

314   minerals, offer snapshots of past environmental conditions, and (ii) the environmental signal refers

315   to the average annual conditions, summer excluded.

316   In the final phase of unit 5 (corresponding to the *Pinus-Betula* pollen assemblage zone PAZ II)

317   an increase in temperature and a tendency toward more arid conditions occurred (Fig. 6).

318   Micromorphological analysis of this laminated sedimentary unit indicated the presence of non-

319   turbulent water during its colluvial deposition (Mücher 2014). These features, in agreement with

320   our results, may relate to periods with less intense rainwash.

321   At the end of PAZ II, the abundance of *Pinus* increased and *Corylus* started to appear (Bakels

322   2014). In unit 6 (PAZ III), *Pinus* declined and *Quercus* started to dominate the vegetation. In

323　addition, *Ilex* and various deciduous trees appeared, suggesting the occurrence of a maritime

324　temperate climate (Bakels 2014). However, the high percentage of herb pollen indicated that,

325　despite the oceanic climatic influence, the environment was still rather open (Fig. 6C; Pop *et al.*

326　2015). The *B. tentaculata* record agreed well with the pollen, indicating a wet climate with periods

327　of more arid conditions (Fig. 6B). Temperatures showed a decrease toward the end unit 6 (Fig.

328　6A).

329　　The few *B. tentaculata* data of unit 7 were not sufficient to achieve a robust environmental

330　interpretation during this phase. According to the pollen record, this part of the succession (end of

331　PAZ III and start of PAZ IV) began with a steady rise in *Corylus* and its subsequent dominance.

332　In general, PAZ IV was characterized by semi-open landscapes with rather scattered forests and

333　some steppe elements (Bakels 2014). During PAZ IVa1 *Ulmus* reached its maximum abundance

334　and algae of the genus *Spyrogyra* overgrew in the lake waters. Furthermore, the percentage of AP

335　(arboreal pollen) versus NAP (non-arboreal pollen) increased (Bakels 2014). This indicated that

336　the climate became more humid, as supported by the decreasing trend in the opercula $\delta^{13}C$ values

337　throughout unit 7 into unit 8 (Fig. 6B). A similar trend was visible for the $\delta^{18}O$ values, suggesting

338　a decrease in temperature (Fig. 6A).

339　　In the first part of unit 8, arboreal species were still dominant (Fig. 6C) and *B. tentaculata*

340　recorded rather high temperatures and humid conditions. In the second part of unit 8, temperatures

341　dropped and a dry event was recorded (only visible at depth 430-435 cm; Fig. 6 A,B).

342　Simultaneously, *Corylus* decreased and non-arboreal vegetation became more abundant (Bakels

343　2014). It is possible that the temperature switch toward cooler conditions may have triggered the

344　decline of the arboreal species. At the beginning of unit 9, the temperatures increased slightly and

345　the humidity level decreased (Fig. 6A,B). From this unit onwards, laminated deposits were

346   replaced by massive deposits with occasional soil formation (Pop *et al* 2015). In this phase, the

347   abundance of *Corylus* remained low (Bakels 2014). Very few mollusc remains were retrieved from

348   PAZ IVb1 (second half of unit 9 and unit 10), suggesting a lake dry up event. Likewise, the shells

349   from unit 11 (PAZ IVb2) indicated that substantial aridity occurred during this period (Fig. 6B),

350   coupled with a rather variable temperature regime (Fig. 6A). Simultaneously, a drop in arboreal

351   vegetation (mainly *Corylus*) was identified by the pollen analyses, together with an increase of

352   steppe-like vegetation (Bakels 2014). Compared to units without aquatic shell remains (e.g. units

353   12 to 16), unit 11 (and units with an aridity signal) indicates that the climate was rather dry but it

354   still allowed the basin to contain water. This hypothesis was also supported by the presence of

355   water plants, *Lemna* pollen and *Spirogyra* (Pop *et al.* 2015, Kuiper 2014). Unit 17 was

356   characterized by the highest $\delta^{18}O$ values of the whole sequence, indicating that the temperatures

357   were significantly higher than before, especially in the lower portions corresponding to the last

358   part of PAZ V. With the start of PAZ VI/VII (end of unit 17), the $\delta^{18}O$ values showed a significant

359   decrease, indicating the occurrence of a cooling trend. A similar tendency was observed in the $\delta^{13}C$

360   values, going from less humid to more humid conditions at the end of unit 17 and beginning of

361   unit 18. The observed elevated temperatures during PAZ V agreed with the reconstruction based

362   on pollen by Kühl & Litt (2003) and the bulk carbonate $\delta^{18}O$ record from Gröbern (an Eemian lake

363   ~ 70 km north-east to NN2) by Litt *et al.* (1996). Indeed, these studies suggested that the

364   temperatures reached a maximum during PAZ IVb and they remained rather stable during PAZ V.

365   Interestingly, the drier conditions observed in the *Bithynia* record from unit 11 (PAZ IVb2) do

366   not seem to be reflected in the pollen sequence of NN1 (Neumark-Nord 1), the larger lake located

367   about one hundred meters away from NN2 (Seifert 1990). Previous studies hypothesized that the

368   absence of such environmental change at NN1 might have been related to either a very small-scale

369   local change or to a bias introduced by the small size of the NN2 lake (Bakels 2014). Indeed, small

370   lakes may catch pollen from a restricted area around the lake itself (Tauber 1967). Furthermore,

371   droppings from animals coming to the lake might have altered the composition of the pollen,

372   enriching the consumed plant species (Bakels 2014). However, the aridity signal from the mollusc

373   opercula confirms that the pollen data were robust and the animal droppings did not significantly

374   affect the vegetation reconstruction. The absence of the *Corylus* drop in NN1 might be related to

375   the different sizes of the two basins. Being a much smaller lake than NN1, NN2 may possibly have

376   been more sensitive to changes in temperature and humidity levels. Therefore, the interpretation

377   of pollen and opercula data from HP7 at NN2 might describe fairly localized environmental

378   conditions and its application to a broader regional level might be challenging.

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380

Implications for Neanderthal presence at NN2

382   Traces of human presence at NN2 were found from unit 6 to unit 17 (Fig. 6; Table 1; Gaudzinski-

383   Windheuser *et al.* 2014; Pop 2014). Our reconstruction indicated that the local environmental

384   conditions changed within these phases, suggesting that Neanderthal groups were present at NN2

385   under varying temperature and humidity regimes. This correlates with the results from the oxygen

386   isotope analysis of horse tooth enamel from the main archaeological find levels in a recent study

387   (Britton et al. 2019). The major archaeological assemblage, with the highest concentration of finds,

388   was NN2/2b (unit 8; PAZ IVa2). The reconstructions based on *B. tentaculata* opercula indicated

389   NN2/2b was the most humid phase of the whole succession (Fig. 6B). High humidity potentially

390   supported the expansion of forested areas, suggesting that semi-closed environments may have

391   occurred during this period. In support of our data, the percentage of arboreal pollen, (especially

392   in the first half of unit 8), was particularly high compared to non-arboreal pollen (Bakels 2014).

393   This also correlates with evidence for niche partitioning between horses and bovids in this phase,

394   which suggested a mosaic and semi-forested environment (Britton et al. 2012). Besides NN2/2b,

395   less dense archaeological assemblages were found at various different stratigraphic depths (Fig. 6;

396   Table 1). The corresponding temperature estimations ranged from very warm (NN2/1a), cold

397   (NN2/3), average (NN2/2a) and variable (NN2/1c; Fig. 6A; Table 1). Similarly, the humidity range

398   was rather broad with very dry (NN2/1c), average (NN2/2a) or with variable conditions (NN2/3,

399   NN2/1a; Fig. 6B; Table 1). Virtually no *B. tentaculata* opercula were recovered from levels

400   NN2/1b and NN2/2c. Therefore, their respective palaeoenvironmental reconstructions are missing.

401   Our results suggested that Neanderthals were present both during the occurrence of relatively

402   dense forests, semi-open environments, as well as more open steppe-like landscapes around the

403   basin. Our data did not indicate any evident environmental preference that might have affected the

404   presence of Neanderthals during the Eemian Interglacial here, which is in agreement with similar

405   recent studies at Neumark (Britton et al. 2019). The character of the presence of Neanderthal

406   groups in central Europe during the Last Interglacial has been the topic of several previous studies

407   (Gamble 1987; Roebroeks *et al.* 1992; Gaudzinski 2002; Richter 2006; Wenzel 2007; Gaudzinski-

408   Windheuser & Roebroeks 2011). In the past, some researchers argued that Neanderthals might

409   have had limited adaptive abilities to survive successfully in fully forested interglacial conditions

410   (Roebroeks & Tuffreau 1999). However, in Central Europe, Neanderthals show a high flexibility

411   in subsistence strategies in a variety of habitats (Gaudzinski-Windheuser & Roebroeks 2011 and

412   references therein; Gaudzinski-Windheuser *et al.* 2018), indicating that they were highly

413   responsive to climatic and environmental shifts (Gaudzinski 2002, 2004; see also Antoine *et al.*

414 2006, and Sier *et al.* 2015 for comparable evidence from north western France). These inferences

415 are in line with the results obtained by this new proxy for palaeoenvironmental reconstructions.

416

417

Conclusions

419 The present study presents the reconstruction of palaeoenvironmental conditions occurring during

420 the Last Interglacial at Neumark-Nord 2 (NN2), Germany. Oxygen and carbon stable isotopes of

421 *B. tentaculata* opercula were used as environmental proxies. Modern specimens were analysed for

422 calibration purposes and the results suggest that the opercula are generally formed throughout the

423 year, with a minor slowdown during summertime. Therefore, the water properties (i.e. temperature

424 and moisture regimes), encoded in the opercula bulk isotope signal in form of $\delta^{13}C$ and $\delta^{18}O$ values,

425 encompasses annual averages with potential exclusion of the warmest period. The isotope data

426 from the NN2 opercula indicate the local conditions during the Last Interglacial were characterized

427 by alternations between warm and cold conditions as well as successions of phases with different

428 humidity levels, although indicate that the absolute values measured were influenced by local

429 hydrological conditions in the lake. The major evidence of human activity, related to Neanderthals,

430 coincides with a humid phase and potentially with a semi-closed type of vegetation. However,

431 human presence was detected in six additional levels within NN2 which were characterized by

432 rather variable environmental conditions. In the light of the results presented in this study, and

433 other recent analyses, we conclude that the local Neanderthal groups in NN2 area did not manifest

434 a clear environmental preference, and instead were active across a wide range of temperature and

435 humidity conditions.

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437

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direction and planning. E. P., W. K. and W. R. gave access to the samples analysed in the project.

S. M. took the lead in writing the manuscript. All authors provided critical feedback and helped

shape the research, analysis and manuscript.

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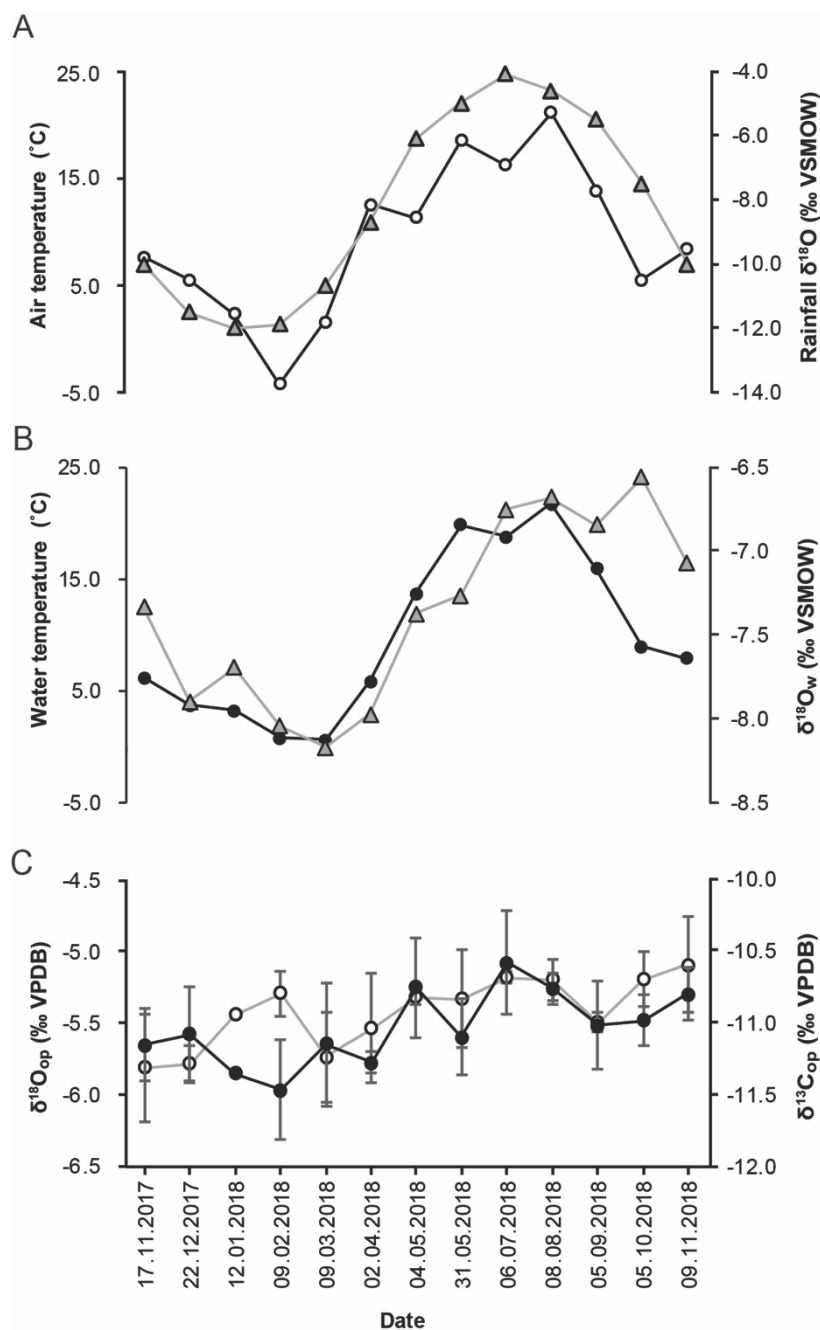622   experimental conditions. *Helgolaender Meeresuntersuchungen 51*, 417–431.

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625

626   Figure and table captions

627

628 **Fig. 1** Location of the modern *B. tentaculata* collection site (Hundewasser: 51°22′37.3″ N,

629 12°16′50.7″ E) and the archaeological site of Neumark-Nord 2 (NN2: 51°19′28″ N, 11°53′56″ E).


630

631 **Fig. 2** A. Air temperatures (open circles) measured at Hundewasser and modelled monthly rainfall

632 $\delta^{18}O$ values (grey triangles and secondary y-axis) obtained from OIPC. B. Water temperature

633 (black circles) and $\delta^{18}O$ values (grey triangles and secondary y-axis) measured throughout the

634   annual collection in Hundewasser. C. Modern *B. tentaculata* opercula (N = 37) δ^{18}O (open circles)

635   and δ^{13}C values (black circles and secondary y-axis). Error bars indicate standard deviation.

636
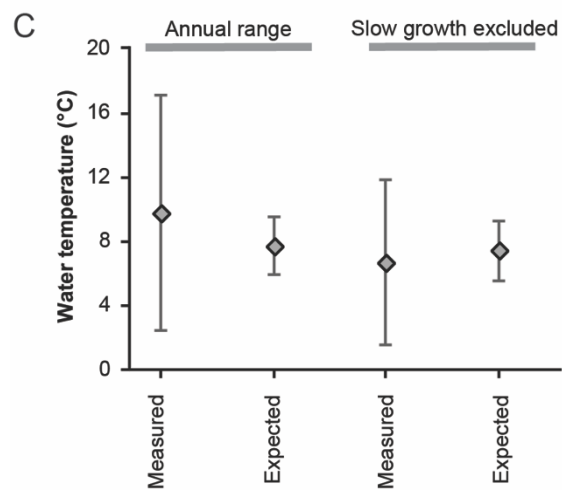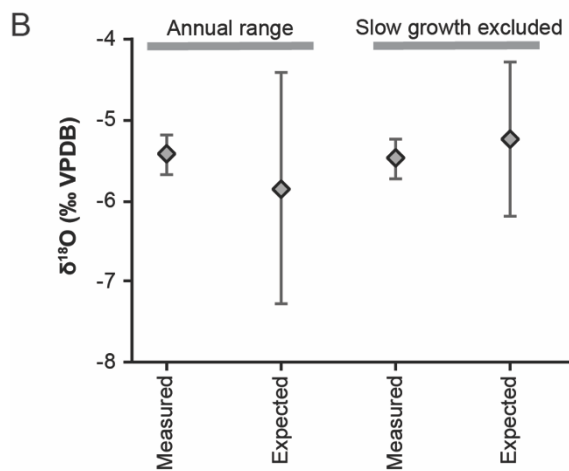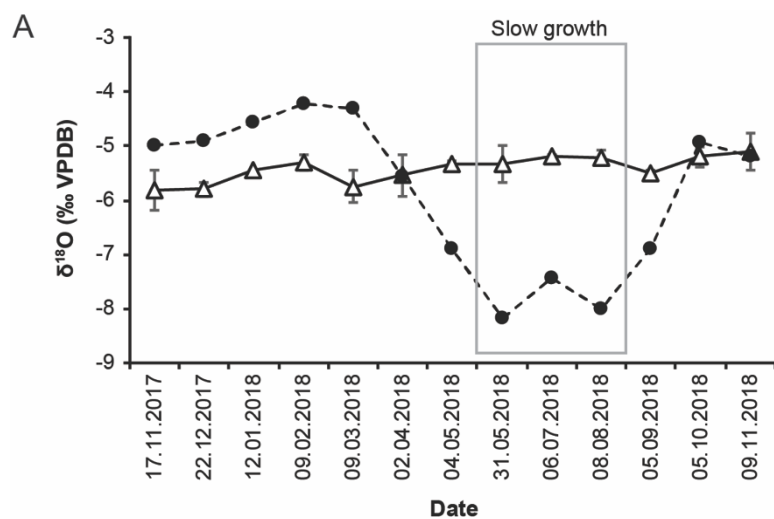
637 **Fig. 3** Modern *B. tentaculata* $\delta^{18}O$ data. A. Monthly opercula $\delta^{18}O$ values measured throughout

638 the annual collection in Hundewasser (open triangles) and the respective expected values (circles)

639 calculated using Eq. (1). The highlighted area represents the potential period of

640 cessation/slowdown of the opercula growth. B. Average measured and predicted values of the

641 opercula $\delta^{18}O$ calculated using the inverse function of Eq. (1). For this purpose, the calculations

642 were carried out using both the whole annual dataset (i.e. 13 measured water $\delta^{18}O$ values and

643 temperatures) and the partial dataset excluding the period of slow growth (i.e. using 10 water and

644 temperature datapoints except the data from June, July and August). C. Average measured and

645 predicted (Eq. (1)) values of the water temperature using the whole annual dataset and the partial

646 dataset excluding the period of slow growth. Error bars indicate standard deviation.
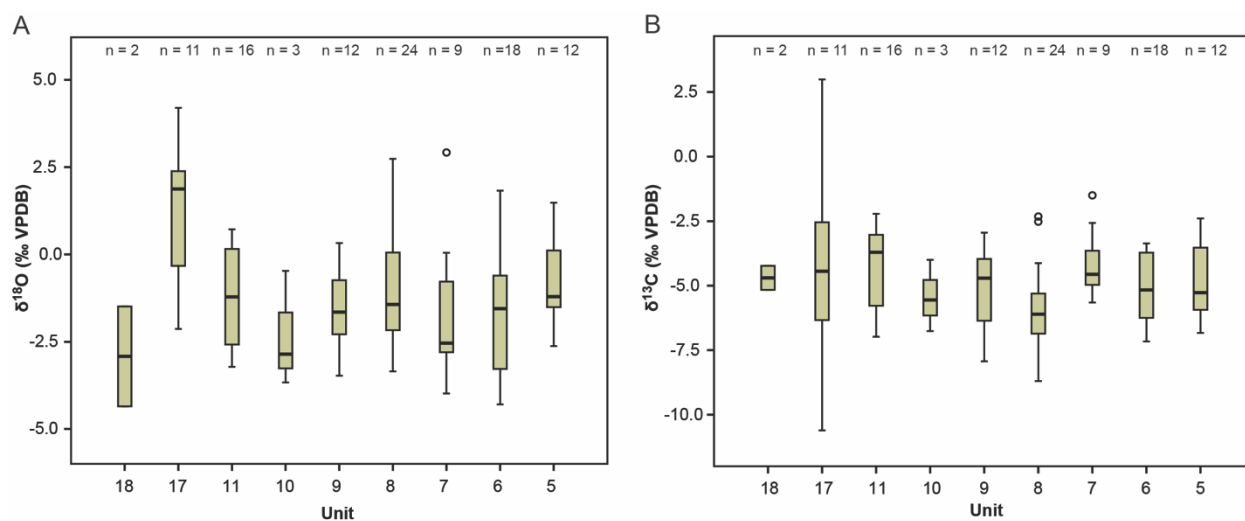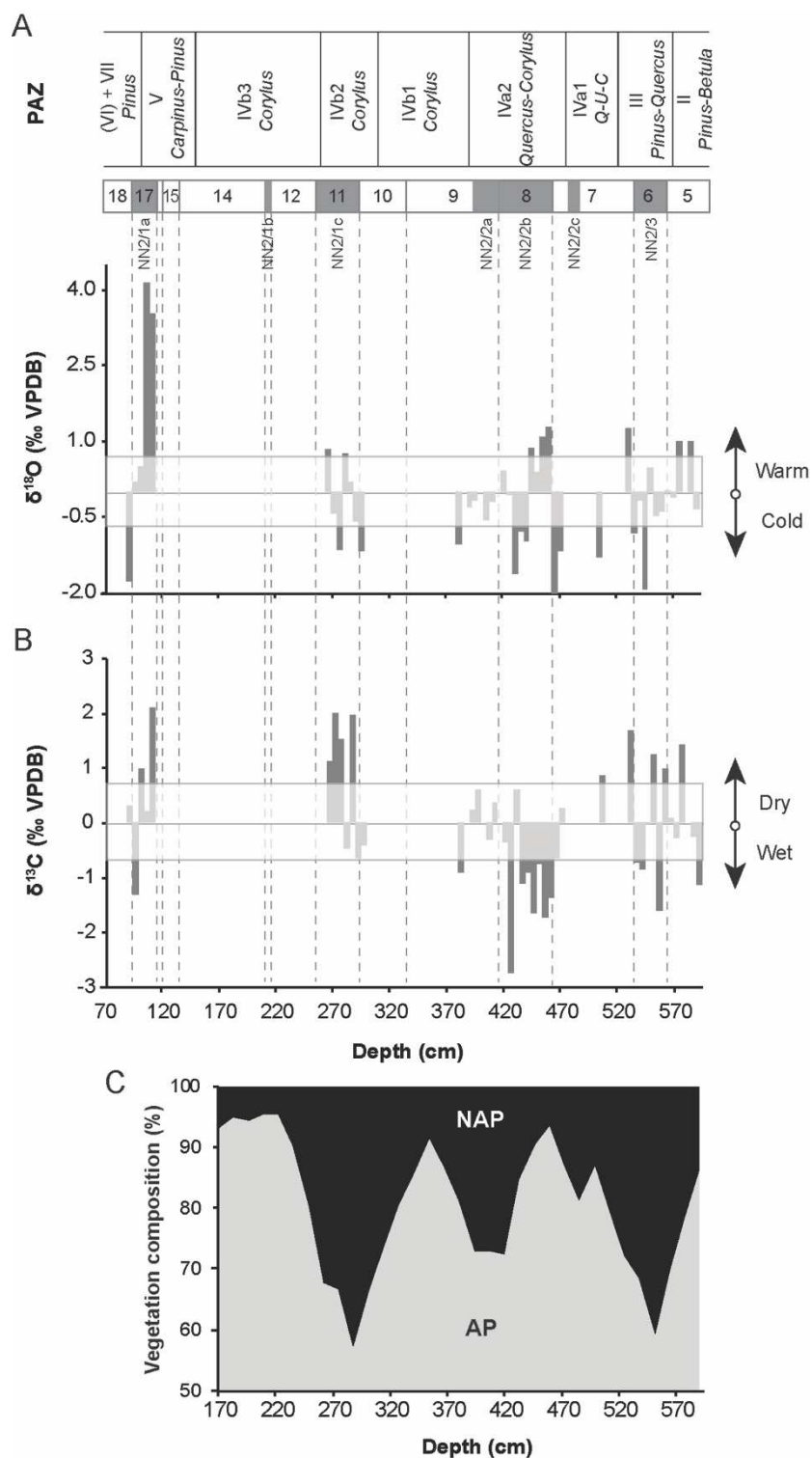

648 **Fig. 4** Overviews of the stable oxygen (A) and carbon isotopes (B) data obtained on *B. tentaculata*

649 opercula from NN2, grouped by stratigraphic units (18 to 5).

33
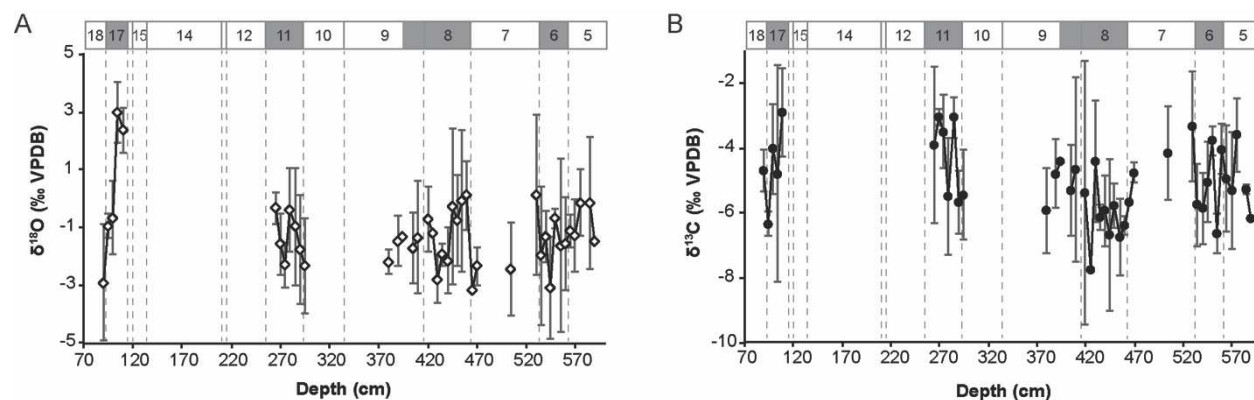
Fig. 5 Raw stable oxygen (A) and carbon isotope (B) data obtained on *B. tentaculata* opercula throughout the NN2 stratigraphic sequence. The bars above the two graphs represent the subdivision of the succession in 14 units (18 to 5). The units in grey contain archaeological findings.

Fig. 6 Stable oxygen (A) and carbon isotope (B) anomalies throughout the different stratigraphic units (18 to 5) and PAZ (Pollen Assemblage Zones; VII to II). Grey areas mark the significance level of 0.7‰ representing the annual isotopic variability. Data outside these envelopes were

659   considered significant for the palaeoenvironmental interpretation. C. Pollen diagram from Pop &

660   Bakels (2015) showing the percentages of arboreal pollen (AP; using the three key taxa of *Pinus*,

661   *Corylus* and *Carpinus*) versus non-arboreal pollen (NAP; including grasses and sedges). *Q-U-C =*

662   *Quercus-Ulmus-Corylus*.

663

Table 1. Find levels at NN2 with corresponding palaeoenvironmental conditions reconstructed from *B. tentaculata* geochemistry record.

Find level	Corresponding unit	Finds m^{-2}	Total finds	Palaeoenvironmental reconstruction	
NN2/3	6	68	271	cold	variable
NN2/2c	7	11	2296	n/a	n/a
NN2/2b	8	255	125,322	warm than cold	very humid
NN2/2a	9	3	1501	average	average
NN2/1c	11	37	8811	variable	very dry
NN2/1b	13	17	1250	n/a	n/a
NN2/1a	17	1	4	very warm	dry than wet

664

665   **Table S1.** Modern environmental data and *B. tentaculata* isotope values during the annual

666   collection conducted at Hundewasser. SD = standard deviation.

667
668
669   **Table S2.** *B. tentaculata* opercula isotope values throughout the HP7 profile in NN2. SD =

670   standard deviation.

671